Exploring if Eyespot Tests can Replace Cognitive Judgement Bias Tasks when Assessing Affective State in Red Junglefowl chicks

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1 Abstract

We can describe cognition as the mental processes involved when processing signals and information from our surroundings. Despite being vital for our actions, these processes can be biased by emotions, which results in a judgement bias of ambiguous information. Depressed individuals tend to be pessimistic about such ambiguous information, while individuals under normal or good condition, tend to be optimistic. This is true also for animals. Based on this, cognitive judgement bias tests are developed to measure the affective state of individuals. However, cognitive judgement bias tests require extensive pre-test training for animals to learn positive and negative reference cues. An alternative to using responses to pre-learnt cues could be to use naturally aversive stimuli instead. Eyespot patterns on lepidopterans can be aversive to birds. However, it is scarcely investigated if eyespot patterns can be used to measure affective state. The aim of my study was therefore to investigate if eyespots patterns can replace classic cues in cognitive judgment bias tests measuring affective state. I did so by comparing behavioural responses of red junglefowl chicks (Gallus gallus) to both eyespot patterns and classical cues in a cognitive judgement bias test. Responses correlated between some cues in the two tests, suggesting that eyespot patterns may work as a replacement of pre-learnt cues. However, no differences in responses to the eyespot patterns was found, and so further work is needed to improve the design of eyespot cues to obtain a clearer correlation between responses to eyespot patterns and classical pre-learnt cues in cognitive judgement bias tests. As less training is needed, such improved tests could have positive implications, and be a simpler and more user-friendly way to measure affective state in animals.

2 Introduction

To be able to take in and process information from our surroundings, and make decisions based on this information, we rely on mental processes referred to as cognition (Gleichgerrcht et al., 2010). Cognition is needed for individuals to make decisions about future events in order to avoid negative outcomes and instead gain rewards (Mendl et al., 2009; Sharot, 2011). Considering how important it can be to make accurate decisions about future events, one would expect the brain to make unbiased decisions (Sharot, 2011). However, how an individual interprets information can in fact be biased and affected by its emotional state (Harding et al., 2004; Mendl et al., 2009).

Such bias is called a cognitive judgement bias and occurs when an individual's previous positive or negative experiences affect their ability to interpret new ambiguous stimuli (Eysenck et al., 1991). Research shows that individuals suffering from depression or anxiety often make a more pessimistic judgement when faced with ambiguous cues (Eysenck et al., 1987; Harding et al., 2004). This is not only true for humans but also for other taxa, as several studies have shown using cognitive judgement bias tests. Such tests are used to assess the level of optimism and pessimism in animals, by for example using ambiguous cues, intermediate between pre-learnt rewarded and unrewarded colour cues (Harding et al., 2004; Bateson & Matheson, 2007). If responses are closer to the pre-learnt negative (unrewarded or punished) cue, animals are considered more pessimistic and if responses are closer to the pre-learnt positive (rewarded) cue, the individual is considered more optimistic. In the original study using this model, rats, Rattus sp., had been pre-trained to press a lever when they heard a tone associated with a positive experience, and to not press the lever when they heard a tone associated with a negative experience, in this case an electric chock (Harding et al., 2004). After training, rats were housed in two different environments, either in familiar conditions or in more stressful conditions (Harding et al., 2004). The latter condition placed rats in a more depressed state of mind. This condition could entail alterations to living conditions such as bedding being left damp, or the light cycle being reversed. Control rats were kept in the same familiar conditions as during training. After nine days in these housing conditions, rats were exposed to unfamiliar tones that were intermediate between the two tones that they had learned during training. Rats with an induced depressed state responded less to ambiguous tones close to the rewarded tone and when responding, doing so slower than rats in neutral housing, indicating lower optimism (Harding et al., 2004). In a follow up study, European starlings, Sturnus vulgaris, were housed in standard and enriched cages (Bateson et al., 2007). Birds were trained to associate a white cue with a palatable food and a dark grey cue with an unpalatable food (Bateson et al., 2007). When these reference cues were learned, birds were presented with three ambiguous cues of grey. Here, birds that were moved from enriched to standard housing conditions before testing were more prone to interpret the ambiguous cues as giving a negative outcome. Both studies show that animals interpret ambiguous stimuli with less optimism when placed in poorer or more stressful conditions, interpreted as

individuals being in a more depressed state (Harding et al., 2004; Bateson et al., 2007).

The ability to assess the affective state in animals objectively is important in animal welfare science (Brilot et al., 2009, Lagisz et al., 2020). This to be able to improve animal welfare by measuring both negative and positive welfare and act to improve it. Cognitive judgement bias tests are commonly used to assess affective state, and is currently our best tool to do so (Lagisz et al., 2020). However, cognitive judgement bias tests require a lot of pre-test training for the study animals to learn the needed reference cues and can cause both stress for the animals and be time consuming (Brilot et al., 2009). This can make cognitive judgement bias tests difficult to apply in situations that call for a welfare assessment that is rapid and kept at a low cost (Brilot et al., 2009). Another potential problem with cognitive judgement bias tests is that test animals can, as test trials go on, learn that ambiguous cues are not rewarded (Bethell, 2015). This can make the tests less efficient in situations that require frequent testing. Therefore, to develop methods for objectively assessing affective state in animals that avoid the drawbacks associated with current cognitive judgement bias testing, researchers have looked at using eyespot patterns as a stimulus instead (Brilot et al., 2009). Eyespot patterns are patterns that resemble vertebrate eyes, and are found to be naturally aversive stimuli. Such patterns are common on for example several lepidopterans, and act to prevent bird attacks. This means that less or no training would be needed when using eyespot patterns as a cue to associate with a negative outcome (Brilot et al., 2009).

Previous studies have shown that eyespot patterns on butterflies have an aversive effect on domestic fowl, *Gallus gallus domesticus* (Olofsson et al., 2012; Olofsson et al., 2015). Based on these findings, I wanted to investigate if responses to eyespot patterns can be used to replace classical pre-learnt cues in a cognitive judgement bias test when assessing affective state in fowl. If so, it would enable to develop a simpler test with less pre-test training needed. I investigated this in a population of red junglefowl, the wild ancestor of domesticated chickens, exposed to both eyespot patterns, and a classical cognitive judgement bias test.

3 Method

3.1 Study population

All data for this study was collected in November of 2017 by Hanne Løvlie and colleagues and made available for me to analyse in 2020. For both the eyespot test and cognitive judgement bias test a study population of red junglefowl (n_{female} = 18, n_{male} = 21), kept at Linköping university, was used. Both the eyespot test and cognitive judgement bias test was conducted when birds were approximately eight weeks old. The study population was housed in three groups with mixed sex in pens with a light:dark cycle of 12:12 hr. Birds had access to water, food, sawdust, and perches ad libitum. Prior to tests, birds had been familiarized with being handled by humans and to be alone in the test arena. Individuals were ID marked with wing tags and tested individually.

3.2 Eyespot methods

To test responses to eyespot patterns, an arena (76 x 114 cm) was used. To make it possible to test two birds at the same time, the arena was divided was divided by a barrier in the middle. During the test a control cue (0, fig 1) with no markings and four eyespot cues (1-4, fig 1) were used, where cues 1-3 were intermediate cues between cues 0 and 4. The cues were printed in black on transparent paper and placed in the same way on the arena floor as to look like a pair of eyes. Due to the floor of the test arena being made from brown cardboard the eyespot patterns on the cues appeared to be black and brown.

3.2.1 Pre-test training for eyespot tests

Before the eyespot test, birds had to undergo pre-test training to make sure that they would approach the eyespot cues in the test arena. This was done by using only the control cue with a reward (half a mealworm) placed in the centre of the cue. Birds successfully finished the pre-test training when they approached and ate the mealworm within 60 seconds from being placed in the arena, without showing signs of distress, three consecutive times. Training sessions were kept to approximately 15 minutes, but could be ended early if the bird showed signs of stress or appeared unresponsive. After approximately one hour of rest, training would continue.

3.2.2 Eyespot testing

To avoid any potential order effects and to make sure to get initial responses to all four cues, birds were divided into four groups of approximately 9-10 birds per group, and were exposed to cues in different orders. Each group had an even sex ratio and saw one of cues 1-4 first (fig 1). To let the birds recover from any potential response to a cue, birds always experienced three trials of control cues (cue 0, fig 1) between experiencing cues 1-4. Only one cue was presented at each trial.

The test started with three trials with the control cue, to make sure that each bird had not forgotten its pre-test training. The following order of cues depended on each group and had a design that was semi-randomized so that all possible pair combinations of cues were included. Timing ended when the bird ate the worm, and if it took the bird more than 60 seconds to eat the worm, latency was recorded as '60'. In both the pre-test training and the test, a trial started when the chick was placed in the test arena and the test observer let go of the bird. At the end of the test, each bird had experienced all four cues three times except for the first cue they experienced, which they experienced a total of four times.

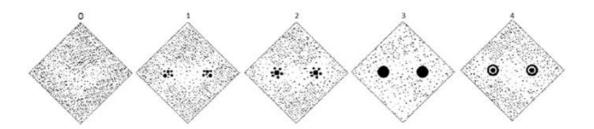


Fig 1. Eyespot patterns used to measure behavioural responses of red junglefowl. Eyespot patterns that birds were exposed to with cue 0 being the control with no visible eyespots, and cue 4 having full eyespot patterns. Cues 1-3 are intermediate patterns of eyespots in between cue 0 and 4.

3.3 Cognitive judgement bias test

The Løvlie group recently developed a visual cognitive judgement bias test, successfully measuring behavioural responses to intermediate, ambiguous cues, in domestic and red junglefowl (Sorato et al., 2018; Zidar et al., 2018; Garnham et al.,

2019). The same type of experimental setup developed by the Løvlie group was used in the cognitive judgement bias test for this thesis.

3.3.1 Pre-test training for cognitive judgement bias tests

After the eyespot test, a judgement bias test was conducted on the fowl to assess their level of optimism. Birds underwent pre-test training where black and white bowls were used as cues (Zidar et al., 2017; Sorato et al., 2018; Zidar et al., 2018; Garnham et al., 2019). The white cue was used with a reward of 1/3 mealworm and the black bowl was unrewarded. The bowls (5 x 3 cm, Ø x H) were placed against the wall of the test arena (50 x 90 x 60 cm, W x H x L). A laminated card (3 x 3 cm) matching the cue (black or white) was placed behind the bowl. Both cues were presented to the bird at the same time with an opaque divider between the cues. When a bird was placed in the test arena timing started, and it ended with the bird either leaving the arena or picking a cue, meaning it approached the cue and had its head at a maximum of 2 cm from it. The sides for which the black and white cues were presented, was alternated during the test in a pseudorandom order. This was done to keep birds from developing a side preference. A bird was considered having successfully finished pretest training when it picked the rewarded cue in six consecutive trials.

3.3.2 Cognitive judgement bias testing

During test trials, a bird was presented with the black (unrewarded) and white (rewarded) cues from the pre-test training in addition to three ambiguous, unrewarded cues of grey (25% black/75% white, 50% black/50% white, 75% black/25% white), in a pseudorandom order, one cue at a time (see Zidar et al., 2017; Zidar et al., 2018; Sorato et al., 2018; Garnham et al., 2019). As during training, cues were made up of a bowl (5 x 3 cm, Ø x H) and a laminated card (3 x 3 cm) matching the colour of the bowl, placed against the wall of the test arena. Each bird experienced the pre-learnt black and white cues 12 times each during the test, and the three ambiguous cues three times each. The latency to approach each cue was recorded in seconds as a measurement of a bird's optimism towards ambiguous cues (since resembling responses to rewarded cues). If a bird did not approach a cue within 30 seconds it was given a latency of '30'.

3.4 Statistical analyses

Statistical analyses were performed using R version 3.6.1 (R Core team, 2019) and SPSS (version24, IBM). Non-parametric analyses were used due to the data not being normally distributed.

To investigate if there was a statistically significant difference between all responses to cues in the eyespot test and the cognitive judgement bias test, a Friedman test was performed on the mean latencies for the study population to approach each cue in both the eyespot test and the cognitive judgement bias test.

A Wilcoxon signed ranks test was also performed on the mean latencies for the study population to approach each cue in the eyespot test to investigate if there were any statistically significant differences between responses to any two cues in the test.

To analyse if responses to eyespot patterns and responses to ambiguous (i.e., grey cues) in the cognitive judgement bias test were correlated, mean latencies of each of the three times the ambiguous cues were presented for each bird to approach every cue in both the eyespot test and the cognitive judgement bias test were compared in a correlation matrix using Spearman rank correlations.

4 Results

When comparing responses to all cues used in the eyespot test, I did not find a statistically significant difference between responses to cues in the study population $(\chi^2(4) = 2.072, p = 0.72, \text{ figure 2})$, however, when comparing responses to all cues used in the cognitive judgement bias test, I found a statistically significant difference between responses to cues $(\chi^2(4) = 96.965, p < 0.001, \text{ figure 3})$.

When comparing responses to cues used in the eyespot test, two cues at a time, I found a statistically significant difference between eyespot cue 0 and eyespot cue 3 (Z = -2.037, p = 0.042, figure 2).

When comparing the mean latencies to approach the cues in the eyespot test and the cognitive judgement bias test, I found a significant correlation between the medium grey cue (50%) in the cognitive judgement bias tests and eyespot cue 3 ($r_s = 0.32$, p =

0.05, figure 4). All other comparisons showed no significant correlation ($r_s < 0.26, p > 0.12$).

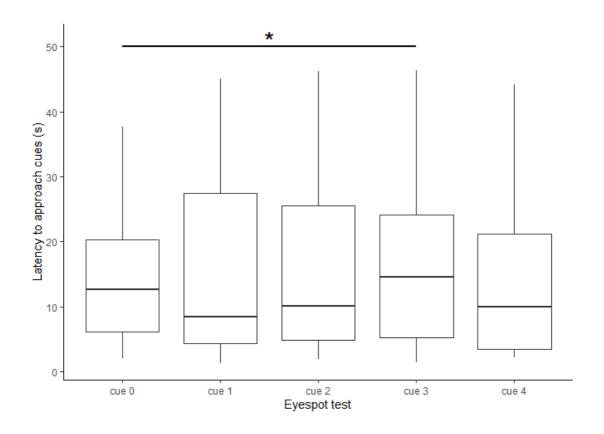


Fig 2. Responses of Red junglefowl chicks to cues used in the eyespot test. Responses to all cues used in the eyespot test (see figure 1). Latencies are shown in seconds. Significant differences are indicated by (*)p < 0.1, $*p \le 0.05$, **p < 0.01, **p < 0.001. Note that the significant result shown is between responses to cue 0 and cue 3 in the eyespot test.

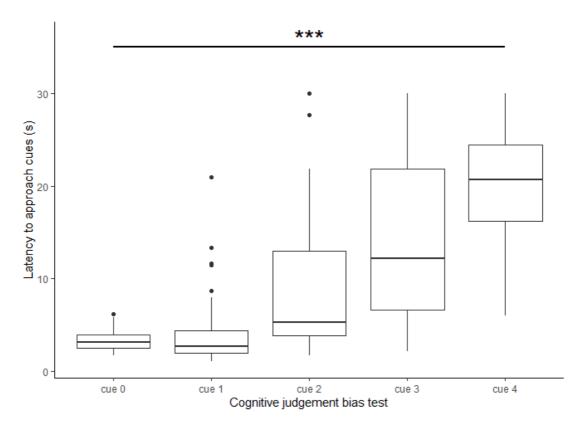


Fig 3. Responses of Red junglefowl chicks to cues used in the cognitive judgement bias test. Responses to all cues used in the cognitive judgement bias. Latencies are shown in seconds. Outliers are shown as dots. Significant differences are indicated by (*)p < 0.1, $*p \le 0.05$, **p < 0.01, ***p < 0.001. Note that the significant result shown is between responses to all cues in the cognitive judgement bias test.

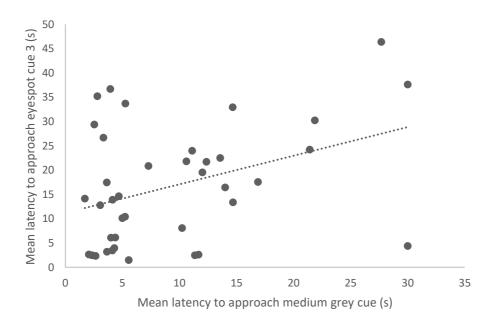


Fig 4. The relationship between responses of Red junglefowl chicks to eyespot- and cognitive judgement bias tests. Responses to eyespot cue 3 (see fig. 1) in the eyespot test and the medium grey cue in the classical cognitive judgement bias test correlated positively. Latencies are shown in seconds. Each dot represents an individual.

5 Discussion

The aim of this study was to explore if behavioural responses to eyespot patterns capture variation in affective state, as measured by a classical cognitive judgement bias test. If so, this could open for eyespot patterns to replace pre-learnt colour-cues used in classical cognitive judgement bias tests when assessing the affective state of animals. I explored this by using individuals from a population of captive red junglefowl exposed to both types of cues.

To do so, I tested whether behavioural responses in an eyespot test, performed with intermediate, ambiguous eyespot patterns as cues, correlated to behavioural responses to ambiguous grey colour cues (intermediate between pre-learnt black and white cues) in a classical cognitive judgement bias test developed for fowl. I started by analysing whether there was a significant difference between responses to all cues within a test, in both the eyespot test and the cognitive judgement bias test, I found that the cues used in the eyespot test did not generate any strong differences in responses compared to the control cue on a group level, however, the responses to cues used in the cognitive judgement bias test showed a strong significant difference. However, when comparing responses to two cues at a time, not compensating for repeated observations, results indicated a difference in responses to the control cue and one of the ambiguous cues in the eyespot test. The same ambiguous eyespot cue was also found to positively correlate with one of the ambiguous grey cues in the cognitive judgement bias test.

Overall, my findings suggest that responses to eyespot patterns can, with modifications done to the eyespot cues and design of the eyespot test, capture what is measured in the classical cognitive judgement bias test, suggesting that the use of eyespot patterns could replace pre-learnt cues in cognitive judgement bias tests when assessing affective state in animals. This is suggested because the responses individuals showed to the cues that correlated positively were similar, and thus describe the same underlying state.

Future work should investigate if stronger differences in responses to cues in the eyespot test can be found by for example changing the test design so that eyespot cues are placed on the far wall of the test arena, instead of on the floor as was done in the eyespot test performed in this study. It could also test whether the design of the

eyespot cues can be altered to generate stronger differences in responses. In a study on the deterring effect of eyespots of a peacock butterfly on naïve adult fowl, researchers used live butterflies with visible eyespots and eyespots painted over, and found that although most birds fled from the butterfly when it started its display, birds confronted with a butterfly with visible eyespots took longer to return to the butterfly (Olofsson at al., 2012). So, to further investigate possibly stronger differences in reactions to the cues in the eyespot test, eyespot cues could in future work also be made to look more life-like by for example being presented on a shape similar to a butterfly, or being sham-painted on the wings of a live butterfly.

If stronger differences between responses to cues in an eyespot test can be obtained, future work could also replicate my work, for example by the use of a simpler design, focusing on the eyespot cues to which birds show a strong difference in response to compared to the control cue in the eyespot test. This could be done by presenting these eyespot cues, and again comparing responses to the ambiguous grey cues in the cognitive judgement bias test, to evaluate if the correlation I observed is still detectable. If this shows the same response pattern, a much simpler test can be used, focusing on responses to these ambiguous eyespot cues, with less or no previous training needed for the animals. However, one should keep in mind that such a test using eyespot patterns as stimuli will probably only work for study animals that have natural predators with these types of eyes, such as birds and rodents. Thus, further work would be needed to find alternative naturally aversive stimuli to be used for other animal groups in order to successfully assess affective state across species, in a simpler way than by the classical cognitive judgement bias test.

A possible explanation for not finding differences between the responses to the cues in the eyespot test could be that fowl possibly do not find eyespots patterns in themselves to be strongly aversive or the other cues not ambiguous enough. In a previous study looking at the aversive effects of butterfly's eyespot patterns in naïve adult fowl, researchers found that although slower to return to the zone with the butterfly, most of the birds that were confronted with a butterfly fled from it, regardless of if the butterfly had had its eyespots painted over, or intact (Olofsson et al., 2012). The results suggests that the display of the butterfly was more aversive to the birds than the eyespot patterns alone. In another study testing whether eyespots of

butterflies had a deterring effect on juvenile fowl, the majority of birds were intimidated by eyespots (Olofsson at al., 2015). Even if birds were more intimidated when eyespots were visible and not painted over, it seems that the eyespot patterns were not the only deterring factor (Olofsson et al., 2015). Further, most birds uttered alarm calls, both when confronted with eyespots that were visible and with eyespots that had been painted over, suggesting that both the display of the butterfly and visible eyespots had a deterring effect on birds (Olofsson et al., 2015). The results from these studies seem to indicate that fowl do not only find eyespot patterns in themselves strongly aversive and further studies with the current model of the eyespot methods on a different predator species would be needed to determine if this suggestion is relevant.

In an earlier study investigating whether aversion to eyespots in starlings could be used as a test of affective state (Brilot et al., 2009), birds showed signs of anxiety after being subjected to a starling alarm call and white noise. Further, the study showed that starlings found eyespots aversive. However, this study did not find an interaction between the auditory stimuli and the eyespot patterns used, suggesting that starlings in an anxiety like state did not find eyespots more aversive than controls. To investigate if this is valid also in the experimental setup I used, two study groups could be used, where one group is stressed (by for example altering living conditions, Harding et al., 2004; Zidar et al., 2018) and keeping one group as an un-stressed control. This would enable to investigate if the response to the eyespot pattern to which birds showed a similar response as to the intermediate, ambiguous grey cue in the classical cognitive judgement bias test, is as expected based on if affective state is altered to be more negative. If responses are as expected, this would further validate that responses to eyespot patterns could be used as a simpler test.

If eyespot patterns can be used as stimuli to measure affective state, as my work in some ways indicate, it would mean that less pre-test training would be needed when assessing affective state, saving resources like time and money (Brilot et al., 2009). Using already existing aversive stimuli in tests would also reduce handling of study individuals, which will likely improve welfare, since handling typically causes stress in test animals.

5.1 Conclusions

In conclusion, the result from my work indicates that eyespot patterns can potentially replace pre-learnt cues in cognitive judgement bias tests to measure affective state in test animals. However, eyespot cues in the eyespot test performed for this study did not generate differences in responses in Red junglefowl chicks, thus further work should investigate if a more optimal ambiguous eyespot cue can be found, to which birds in a negative state show a negative response, and birds in a neutral or positive state are unaffected. If varieties of eyespot patterns are more efficient in capturing affective state than classical cues used in cognitive judgement bias tests (e.g., by being more ambiguous), and responses to these eyespot patterns are similar to responses to classical cues used in cognitive judgement bias tests (which is still the most validated and robust test of affective state in animals), eyespot patterns could work in replacing these classical cues, saving valuable resources.

6 Societal & ethical considerations

If it can be shown that naturally aversive stimuli can be used when assessing emotional state in animals, it could save researchers resources, time, and handling of the animals. Being able to assess affective state (both positive and negative) in study animals can help us to improve animal welfare, by for example improving housing for study animals and animals in captivity, and if animals are being handled by humans as little as possible in these tests, this would also reduce stress.

All tests performed in this study include handling of chickens. All tests in this study were carried out by trained researchers that have passed a Laboratory Animal Sciences (LAS) course and tests were approved by Linköping ethical committee (permit number 50-13).

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8 References

Bateson, M., & Matheson, S. M. (2007). Performance on a categorization task suggests that removal of environmental enrichments induces 'pessimism' in captive European starlings (Sturnus vulgaris). Animal Welfare, 16, 33-36. https://www.staff.ncl.ac.uk/melissa.bateson/Bateson_Matheson_2007.pdf

Bethell, E. J. (2015). A"How-To" Guide for Designing Judgement Bias Studies to Assess Captive Animal Welfare. Journal of Applied Animal Welfare Science, 18, 18-42. https://doi.org/10.1080/10888705.2015.1075833

Brilot, B. O., Normandale, C. L., Parkin, A., & Bateson, M. (2009). Can we use starlings' aversion to eyespots as the basis for a novel 'cognitive bias' task? Applied Animal Behaviour Science, 118, 182-190.

https://doi.org/10.1016/j.applanim.2009.02.015

Eysenck, M. W., MacLeod, C., & Mathews A. (1987). Cognitive functioning and anxiety. Psychological Research, 49, 189-195. https://doi.org/10.1007/BF00308686

Eysenck, M. W., Mogg, K., May, J., Richards, A., & Mathews, A. (1991). Bias in Interpretation of Ambiguous Sentences Related to Threat in Anxiety. Journal of Abnormal Psychology, 100, 144-150. https://doi.org/10.10b37/0021-843X.100.2.144

Garnham, L. C., Porthén Ahlgren, S., Child, S., Forslind, S., & Løvlie, H. (2019). The role of personality, cognition, and affective state in same-sex contests in the red junglefowl. Behavioral Ecology and Sociobiology, 73, 1-12. https://doi.org/10.1007/s00265-019-2762-0

Gleichgerrcht, E., Ibáñez, A., Roca, M., Torralva, T., & Manes, F. (2010). Decision-making cognition in neurodegenerative diseases. Nature Reviews Neurology, 6, 611-623. https://doi.org/10.1038/nrneurol.2010.148

Harding, E. J., Paul, E. S., & Mendl, M. (2004). Cognitive bias and affective state. Nature, 427, 312. https://doi.org/10.1038/427312a

Lagisz M., Zidar, J., Nakagawa, S., Neville, V., Sorato, E., Paul, E. S., Bateson, M., Mendl, M., & Løvlie, H. (2020). Optimism, pessimism and judgement bias in

animals: A systematic review and meta-analysis. Elsevier, 118, 3-17. https://doi.org/10.1016/j.neubiorev.2020.07.012

Mendl, M., Burman, O. H. P., Parker, R. M. A., & Paul, E. A. (2009). Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. Elsevier, 118, 161-181. https://doi.org/10.1016/j.applanim.2009.02.023

Olofsson, M., Løvlie, H., Tibblin, J., Jakobsson, S., & Wiklund, C. (2012). Eyespot display in the peacock butterfly triggers antipredator behaviors in naïve adult fowl. Behavioral Ecology, 24, 305-310. https://doi.org/10.1093/beheco/ars167

Olofsson, M., Wiklund, C., & Favati, A. (2015). On the deterring effect of a butterfly's eyespot in juvenile and sub-adult chicken. Current Zoology, 61, 749-757. https://doi.org/10.1093/czoolo/61.4.749

Roelofs, S., Boleij, H., Nordquist, R. E., & van der Staay, F. J. (2016). Making Decisions under Ambiguity: Judgement Bias Tasks for Assesing Emotional State in Animals. Frontiers of Behavioural Neuroscience, 10, 119. https://doi.org/10.3389/fnbeh.2016.00119

Sharot, T. (2011). The optimism bias. Current Biology, 21(23), R941-R945. https://doi.org/10.1016/j.cub.2011.10.030

Sorato, E., Zidar, J., Garnham, L., Wilson, A., & Løvlie, H. (2018). Heritabilities and co-variation among cognitive traits in red junglefowl. Philosophical Transactions of the Royal Society B, 373, 20170285. https://doi.org/10.1098/rstb.2017.0285

Zidar, J., Sorato, E., Malmqvist, A-M., Jansson, E., Rosher, C., Jensen, P., Favati, A., & Løvlie, H. (2017). Early experience affects adult personality in the red junglefowl: A role for cognitive stimulation? Behavioral Processes, 134, 78-86. https://doi.org/10.1016/j.beproc.2016.06.003

Zidar, J., Campderrich, I., Jansson, E., Wichman, A., Winberg, S., Keeling, L., & Løvlie, H. (2018). Environmental complexity buffers against stress-induced negative judgement bias in female chickens. Scientific Reports, 8, 1-14. https://doi.org/10.1038/s41598-018-23545-6